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Short Communication

First Report of *Pseudomonas tolaasii* Causing Bacterial Brown Blotch of White *Hypsizygus marmoreus* in Taiwan

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Abstract

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Hypsizygus marmoreus (Peck) H. E. Bigelow 1976, commonly known as the white elf mushroom (白精靈菇) when cultivated in a sawdust bag, is a commercially important mushroom species in Taiwan. In March 2023, infected samples showing yellow sticky symptoms on the lower parts of stipes were collected from a cultivated farm in Changhua, Taiwan. Bacterial ooze was observed flowing out of the infected stipes when they were suspended in sterile water. The isolated bacterium from the infected fruiting bodies was grown on a nutrient agar (NA) medium, forming circular colonies with smooth surfaces and a milky yellow color. It also exhibited yellow-green fluorescence on King's B medium. To confirm the pathogenicity of the bacterial isolate on white elf mushroom, its suspension in sterile water was sprayed on the surfaces of fruiting bodies, resulting in yellow and water-soaked symptoms within 1–3 d after inoculation. Over time, the symptoms expanded, leading to severe brown blotching and rot of the fruiting body tissues, accompanied by a sticky odor on the 7 d after inoculation. Based on the results of observation of cultural characteristics and pathogenicity tests that conform to Koch's postulates, as well as the result of 16S rRNA sequence analysis, the causal agent of the bacterial brown blotch disease on white *H. marmoreus* was identified as *Pseudomonas tolaasii* Paine. This paper represents the first report of bacterial brown blotch disease on white *H. marmoreus* in Taiwan. In the future, further surveys of the brown blotch disease in different cultivation areas are needed. Additionally, the disease control effect of environmental disinfectants such as hypochlorous acid-containing water will be tested to reduce the economic losses caused by the bacterial disease.

Key words: White *Hypsizygus marmoreus*, Bacterial Brown Blotch, *Pseudomonas tolaasii*.

Mushroom cultivation is of great economic importance in Taiwan; however, it is susceptible to various pathogenic infections that can lead to severe economic losses (Liu *et al*. 2022). Among the commercially cultivated edible mushrooms, white *Hypsizygus marmoreus*, also known as the white elf mushroom (白精靈菇) when grown in sawdust bags, is a popular variety.

In 2023, samples displaying yellow sticky symptoms on the lower parts of stipes were collected from a cultivated field in Changhua, Taiwan. The affected areas exhibited a water-soaked appearance, and some stipes of the mushroom showed brown blotch and rotten areas (Fig. 1A). Upon the infected stipes were suspended in sterile water, bacterial ooze was observed flowing out. The suspension was streaked over nutrient agar (NA) medium (composition: Beef extract 3 g, Peptone 5 g, Agar 15 g, Distilled water 1 L) and King's B (KB) medium (composition: Proteose peptone No. 3 20 g, Glycerol 10 mL, K_2HPO_4

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1.5 g, $MgSO_4$ 1.5 g, Agar 15 g, Distilled water 1L). On the NA medium, most colonies were initially circular with smooth margins and appeared oyster-white in color (Fig. 2A). The bacterium was then purified and isolated through a series of dilutions of the bacterial suspension from a single colony, followed by streaking on KB medium. Moreover, the isolated bacteria exhibited fluorescent pigments on KB medium after 48 h of incubation at 25℃ (Fig. 2B), confirming that the bacterium isolated in this study belongs to the *Pseudomonas* genus.

The pathogenicity test for the isolated bacterium was conducted by spraying the water suspension (approximately 4–5 × 10^8 colony-forming unit (CFU) mL^{-1}) onto the surfaces of healthy white *H. marmoreus* fruiting bodies, while fruiting bodies sprayed with sterilized water served as a negative control. The bacterial suspension was prepared by suspending the isolated bacterium in sterilized water after it had been grown in NB broth at 24℃ for 12 h on a rotary shaker (200 rpm; Orbital Shaking Incubator S300R, FIRSTEK™, New Taipei, Taiwan). A 10–15 mL bacterial suspension was

Fig. 1. Symptoms of brown blotch disease on white *Hypsizygus marmoreus*: (A) sample of infected fruiting bodies collected from cultivated farm located at Changhua, Taiwan; (B) fruiting bodies displaying yellow and water-soaked lesion 3–4 d after being inoculated with *Pseudomonas tolaasii*, and becoming brown blotched with sticky odor at 7 d; and (C) fruiting bodies sprayed with sterile water (negative control).

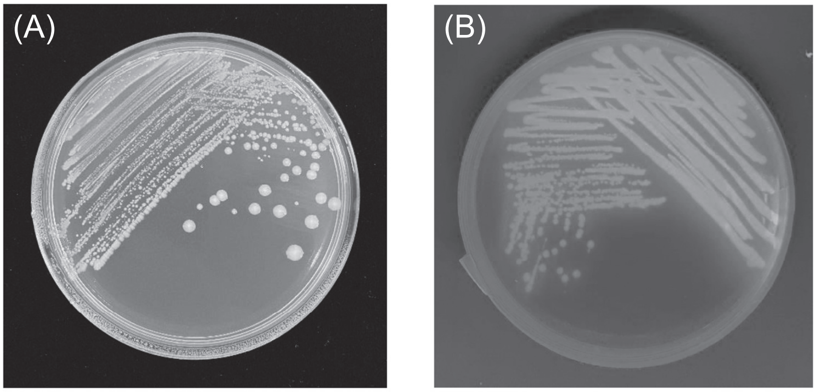

Fig. 2. Colony morphology of *Pseudomonas tolaasii*: (A) *P. tolaasii* cultured on nutrient agar (NA) appeared as white and circular colony; and (B) on King’s B medium, the colonies produced yellow-green fluorescence.

evenly sprayed on each of two tested fruiting body samples, with at least three fruiting bodies in each sample. The inoculated fruiting bodies were then incubated at 13℃ with a relative humidity of 92% for one day. Subsequently, one of the tested samples was maintained at 13℃ with a relative humidity of 92%, while the other sample was transferred to an incubator at 20℃ with the same relative humidity as the former. Assays were conducted two times. Following the inoculation, water-soaked symptoms were observed on the first treated sample 3–4 d after the procedure. The affected fruiting bodies then turned yellow and eventually rotted (Fig. 1B). After 7 d, the inoculated fruiting bodies had become rotten with an unpleasant odor. Similar symptoms were also observed on the inoculated fruiting bodies of the second sample. In contrast, the negative control consisting of healthy fruiting bodies treated with sterilized water remained asymptomatic (Fig. 1C). The results of the pathogenicity test clearly conform to Koch's postulates. Additionally, the 16S rRNA gene of the isolated bacterium was amplified by using universal primers fDI and rDI (Weisburg *et al*. 1991) and sequence analysis via National Center for Biotechnology Information (NCBI) Nucleotide to Nucleotide Basic Local Alignment Search Tool (BLASTn) indicated a high sequence identity with *Pseudomonas tolaasii* (query coverage of 99%, identity of 99.71%, e-value of 0), supporting its preliminary identification as *P. tolaasii*.

P. tolaasii has been reported to cause bacterial blotch diseases in various mushrooms, including *Agaricus bisporus*, *Cordyceps militaris*, *Flammulina filiformis*, *H. marmoreus*, and *Pleurotus ostreatus* in China, leading to brown blotch lesions (Han *et al*. 2012; Osdaghi *et al*. 2019; Liu *et al*. 2022). This study represents the first report of bacterial diseases in white *H. marmoreus* in Taiwan. Further surveys of *Pseudomonas* sp. damages in other *H. marmoreus* cultivated farms in Taiwan are warranted. Additionally, the effectiveness of hypochlorous acid-containing water, and various environmental disinfectant in controlling the pathogen need to be tested to develop an effective disease control strategy.

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首次報導 *Pseudomonas tolaasii* 導致台灣白精靈菇細菌性褐菇病

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摘要

余祥萱、呂昀陞。2023。首次報導 *Pseudomonas tolaasii* 導致台灣白精靈菇細菌性褐菇病。台灣農業研究 72(4):359–362。

白精靈菇是台灣常見的商業化栽培品種之一，以太空包栽培白色鴻喜菇而聞名 (白精靈菇)。於 2023 年 3 月，從彰化縣白精靈菇栽培業者處採集子實體樣本，其菌柄底部表面出現黃褐化、呈水浸狀病徵，並且有細菌菌泥流出。進一步使用營養培養基 (nutrient agar; NA) 與 King's B (KB) 培養基進行分離純化。在 NA 培養基上，所得的分離株呈乳黃色且表面光滑之圓形菌落。而在 KB 培養基上則產生黃綠色螢光色素。經由將所得分離株以噴霧方式重新接種於市售外觀健康的白精靈菇體，結果出現黃白色水浸狀病斑，且病斑隨時間擴大。接種後 7 d，罹病部分出現大面積褐化與腐爛現象，同時散發惡臭，顯示所分離之細菌對白精靈菇具有病原性。進一步鑑定此病原菌，本研究對該菌之 16s rDNA 進行增幅並進行序列分析後，初步鑑定該菌為 *Pseudomonas tolaasii* Paine。依據柯霍式法則與核酸序列分析結果可確認，白精靈菇細菌性褐菇病係由 *P. tolaasii* 所引起。本篇為國內首次報導 *P. tolaasii* 導致白精靈菇細菌性褐菇病之發生，未來將持續調查其他菇場之發生情形，同時也將探討以次氯酸水等環境消毒劑防治本病害之可行性，以找尋本病害之病害管理方法。

關鍵詞：白精靈菇、細菌性褐菇、*Pseudomonas tolaasii*。

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